THE "ACCESSORY CHROMOSOME" OF ANASA TRISTIS.

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In the *Quart. Journ. Mic. Sci.*, Vol. 48, 1905, Professor J. E. S. Moore and L. E. Robinson writing on the spermatogonesis of *Periplanata Americana* claim that the nucleolus of the first spermatocyte is undoubtedly the homologue of the structure described by Paulmier ('99), Montgomery ('01), and McClung ('02), in different forms as one or two of the spermatogonial chromosomes. It's morphological resemblance to a chromosome shown by its frequent elongate form, Moore and Robinson attribute to mechanical influences and claim that normally it is spherical like the nucleolus. In three recent papers Professor E. B. Wilson ('05, '06) has given special attention to this structure in a number of forms and the above interpretation of Moore and Robinson he ascribes to superficial work.

A study of the spermatogenesis of *Anasa tristis*[1] has convinced us that for this form the interpretation of Moore and Robinson is correct, that the nucleolar-like structure of the rest stage is the homologue of the nucleolus of the egg, that it is not a chromosome, as claimed by the three cytologists who have investigated this form.

In 1899 Paulmier identified this body of the rest stage with the two small spermatogonial chromosomes which Wilson has aptly named the "microchromosomes."

Montgomery in 1901 supported Paulmier in this interpretation, but in 1906 he changed his position and now supports Wilson in identifying this structure — the so-called "chromosome nucleolus" — as one of the larger spermatogonial chromosomes an unpaired spermatogonial chromosome, called by Wilson the odd or "heterotropic" chromosome. Both these investigators claim that it divides only in the first division, in the second division, passing undivided to one pole of the spindle.

[1] We are indebted to the courtesy of Dr. P. R. Uhler for identifying our material.

In Wilson's "Studies on Chromosomes, Nos. II. and III.," he has published several text figures of *Anasa tristis*, including the stages from the "contraction phase" of the first spermatocyte to the anaphase of the second spindle, and also four spermatogonial groups in which he figures an odd number of spermatogonial chromosomes, *i. e.*, 10 pairs, and one large unpaired univalent, the odd or "heterotropic" chromosome.

A study of a large number of smear preparations of the testes of *Anasa tristis* has forced us to the conclusion that in the spermatogenesis of this form there is no so-called "accessory chromosome" — no odd, "heterotropic" chromosome — that the so-called "chromosome nucleolus" of the rest stage is the homologue of the nucleolus of the egg, that in its form and time of disappearance it bears a striking resemblence to the plasmosome of the egg of *Allolobophora fœtida*. Our observations and interpretations are so at variance with the conclusions reached by the three cytologists who have studied this form, that we would hesitate to take issue with such competent authority were we not able to support our observations by a very large number of photomicrographs of the preparations. We have already nearly 200 photographs which seem to demonstrate beyond question the following points.

That the so-called "chromosome nucleolus" of the resting spermatocyte is morphologically the equivalent of a nucleolus, that it is not a chromosome. Wilson has emphasized the evidence of morphological likeness in his Fig. 2, *b* and *c* ("Studies on Chromosomes, II."), in which he shows a structure which he interprets as a chromosome and which has a marked morphological resemblance to his sketch of the "chromosome nucleolus" in his Fig. *a*.

It is significant that in Wilson's three figures of this early prophase only 6 of the 11 bivalents are shown in two of his drawings and only 7 in the third. In fact, not one of the investigators of this form has given a single figure of this stage in which *all* the eleven chromosomes are shown.

In our photographs, on the contrary, *all* the 11 bivalents are in evidence, and not one of them resembles in the least the "heterotropic" chromosome figured by Wilson. This holds true for hundreds of cells in which all the eleven bivalents are present and clearly defined.

It is only when the chromosomes or parts of chromosomes are abnormal that they show a condensed chromatin mass, or masses, suggesting a resemblance to a nucleolus. In many cases one of the arms of a cross-shaped chromosome will resemble a round dense nucleolus and this may appear in from one to five of the crosses, and again both arms, or the entire cross may have degenerated into a compact, deeply staining mass of chromatin. We have a number of photographs of connecting stages between these extremes, and they leave no doubt that the normal chromosome resembles in no way a nucleolus.

Our preparations also demonstrate that the "chromosome nucleolus" like the plasmosome of the egg of *Allolobophora fœtida* has disappeared, as a rule, when the chromosomes are formed (early prophase) very rarely persisting until after the chromosomes have attained their definite shape.

Our smear preparations further demonstrate the absence in the resting spermatocyte of any other structure which can be interpreted as a nucleolus. We approached the study of this form with the hope of being able to identify a structure in the male cell which could be interpreted as the homologue of the "accessory nucleolus" of the egg,[1] but we have found no structure sufficiently pronounced or constant to justify our interpreting it as an "accessory nucleolus."

Paulmier ('99), Montgomery ('01) and Wilson ('05–'06) have all indicated a second nucleolar-like structure in the resting spermatocyte which they interpret as the true plasmosome, but we have been unable to demonstrate a second nuleolar-like structure in our smear preparations. However in sections of testes fixed with Hermann's fluid and stained with iron hæmatoxylin and with anilin stains, we often find nuclei of resting spermatocytes in which a second nucleolar-like structure is differentiated, but the complete absence of such a feature in our smear preparations, makes us hesitate to interpret these two structures as the homologues of the plasmosome and accessory nucleolus of the egg.

Again our preparations demonstrate that the so-called univalent "heterotropic" chromosome is distinctly a bivalent. Its constant bivalent character indicates that it represents in value

[1] Foot & Strobell, 1905.

two spermatogonial chromosomes and not one, and when this chromosome is first formed its bivalent character is much more pronounced than at the later prophase stages. Our photographs however support Wilson in his claim that it appears only *exceptionally* as a tetrad — as a rule this and the micro-chromosomes appear bivalent, while all the others show a marked tetrad character. The frequent eccentric position of this bivalent chromosome, outside the characteristic ring arrangement of the chromosomes in the late prophase, seems to warrant suggesting "eccentric" chromosome as a convenient descriptive name for this special chromosome.

Individuality of the Chromosomes. — Our preparations show a marked individuality of the chromosomes, and in this support the observations of Paulmier, Montgomery and Wilson. Several of the chromosomes can often be clearly identified during the prophases, metaphase and anaphase, though a comparison of a large number of photographs demonstrates that the *form* is not constant. For example, at a definite prophase, 7, 8 or 9 of the 11 bivalents may be clear and sharply defined crosses, while again in the same stage we may have all rods or only 1, 2 or 3 crosses, this indicating that the cross type is not invariably associated with any one chromosome.

During the growth period the chromosomes certainly lose their individuality as completely as in the case of *Allolobophora fœtida*, and we have therefore no positive proof that each bivalent of the prophase represents the same chromatin that formed a pair of the spermatogonial univalents. There is a certain degree of constancy in the relative sizes of the chromosomes, although a definite chromosome may differ greatly in size in different cells at the same stage of development. This may be due in some degree to the technique, but this difference is often so great that we feel convinced it is probably due at least in part, to an actual difference in the size of the individual chromosomes.

Montgomery ('06) observed an inequality in the size of the two microchromosomes, but in our preparations we do not find any support for this observation.

Plane of the First Division. — In many cells in which all the

chromosomes are clearly defined a transverse division for *each* chromosome can be plainly demonstrated by one photograph. In many other cells, however, it can be as clearly determined that the "eccentric" chromosome divides longitudinally while all the others divide transversely. It may be stated, as a rule, that the "eccentric" chromosome divides longitudinally, though many exceptions can be demonstrated.

The Lagging Chromosome of the First Division. — At the late anaphase or early telophase of many of the first divisions, it can be demonstrated that one of the chromosomes has divided at a later period than the others. It may be seen between the two poles in all stages of separation — sometimes the entire bivalent will be between the poles, its two univalent halves having just separated or one univalent may have reached one pole while the other half still lies midway between the poles. We have several photographs in which a lagging chromosome is shown while all the other chromosomes can be counted, thus an error in interpretation is quite impossible. As a rule this lagging chromosome appears to be the "eccentric" chromosome, though it cannot be demonstrated that it is invariably the same chromosome which lags in division. We have not found this phenomenon with sufficient frequency to justify our interpreting it as a constant feature of this division. We think the condition exceptional, though not necessarily pathological.

The Lagging Chromosome of the Second Division. — The second division shows a phenomenon which appears to us to be the equivalent of the one just described for the first division. It is more frequently found for the second spindle than for the first and much more difficult to interpret, as these spindles are so exceedingly small and the chromosomes so closely crowded together, that cases are rarely found in which *all* the chromosomes are in evidence and the true value of the lagging chromosome can be safely interpreted. We have a few photographs which we think throw some light upon this point. *All* the chromosomes at each pole are demonstrated and the lagging chromosome lying midway between the poles, in several cases shows a distinct transverse constriction. In one preparation the two halves have separated, and in another the two halves have

reached opposite poles of the spindle, though in these cases the chromosomes at the poles are too crowded to be counted. We interpret this lagging chromosome as a univalent, being equal in value to all the other chromosomes of the second spindle, just as in the first spindle we interpret the lagging chromosome as a bivalent — both cases indicating simply a retarded division of one of the chromosomes.

Our preparations do not support Wilson and Montgomery in their observation that the lagging chromosome goes over undivided to one pole of the second spindle, and we are therefore unable to follow them in supporting McClung's theory of the dimorphism of the spermatozoa.

If these authors are correct in interpreting this lagging chromosome as only half of a univalent, what is the significance of the frequent transverse constriction? What can such a constriction mean but foreshadowing a division? and this interpretation is supported by the cases in which the division of this chromosome is actually demonstrated. We do not interpret the presence of a lagging chromosome in the first or second spindle as necessarily an abnormal condition though it may be a step in that direction, for we have seen unmistakably pathological spindles where sometimes one and sometimes two chromosomes pass to one pole undivided. We have photographs of some of these spindles and their pathological character can be readily recognized. We also have examples of such unequal, abnormal separation of the chromosomes in the first and second spindles of *Allolobophora fœtida.*

Spermatogonial Chromosomes. — Paulmier ('99) who was the first to study the spermatogenesis of this form interpreted the number of spermatogonial chromosomes to be 22 and in his Fig. 9 has reproduced one of his sections in which 22 chromosomes are clearly represented.

Montgomery ('01) supports Paulmier in his estimate of the number of spermatogonial chromosomes and in his Fig. 74 gives a very clear demonstration of this number.

Wilson in his "Studies on Chromosomes, No. 1," corrects this original count of the spermatogonial chromosomes with such positive assurance [1] that we have great hesitation in questioning

his results and would not presume to do so were it not possible to corroborate our observations with photomicrographs in which 22 chromosomes can be counted without any question. We realize in common with all cytologists the difficulty of getting a correct count of so large a number of small bodies crowded into a contracted space. If 2 or more chromosomes are in such close contact that their line of separation is obscured a correct count is impossible. It is certainly possible to find cells in which only 21 chromosomes can be differentiated and still easier to find cells in which only 20 or 19 are defined. It is much more difficult to find each chromosome so distinctly isolated that all can be demonstrated in one photograph.

Wilson ('05–'06) corroborates his original count of 21 spermatogonial chromosomes and illustrates this point in four sketches. Montgomery ('06) in his last paper withdraws his earlier estimate of the number and supports Wilson's results, figuring 21 chromosomes in his sketch No. 151.

In view of this weight of authority we do not feel inclined to be in the least dogmatic in our estimate of the count of the chromosomes, but our preparations certainly justify us in maintaining that it is possible to demonstrate 22 spermatogonial chromosomes in *Anasa tristis*, as we shall show later in photomicrographs of the preparations themselves.

December 1, 1906.

[1] "Since this paper was sent to press I have determined beyond the possibility of doubt, I think, that the number of spermatogonial chromosomes in *Anasa tristis* is 21 not 22 as given by both Paulmier and Montgomery. This result is based on a study of a large number of preparations and careful camera drawings of more than 20 perfectly clear metaphase figures have been made. All without exception show 21 chromosomes, and I have sought in vain for even a single cell that shows 22."

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